FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE / PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **101195-70**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): **10/031152**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE00/02258	**12 July 2000 (12.07.00)**	**15 July 1999 (15.07.99)**

TITLE OF INVENTION

Tissue Regenerating Agent

APPLICANT(S) FOR DO/EO/US

Heinrich Leonhardt; and Christa M. Cardoso

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

    Application Data Sheet

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/031752	PCT/DE00/02258	101195-70

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :	CALCULATIONS PTO USE ONLY
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1040.00	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $890.00	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $740.00	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $710.00	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$890.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	$130.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	- 20 =	0	x $18.00	$0.00
Independent claims	- 3 =	0	x $84.00	$0.00
Multiple Dependent Claims **(check if applicable)**.			☐	$0.00
TOTAL OF ABOVE CALCULATIONS			=	$1,020.00
☒ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				$510.00
SUBTOTAL			=	$510.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	$130.00
TOTAL NATIONAL FEE			=	$640.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	$0.00
TOTAL FEES ENCLOSED			=	$640.00
			Amount to be: refunded	$
			charged	$

a. ☐ A check in the amount of _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **14-1263** in the amount of **$640.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **14-1263** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

correspondence address associated with Customer No. 27387

27387

PATENT TRADEMARK OFFICE

SIGNATURE

Bruce S. Londa

NAME

33,531

REGISTRATION NUMBER

January 14, 2002

DATE

10/031152

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Atty's Docket No. 101195-70

EXAMINER :

GROUP ART UNIT :

APPLICANT : Heinrich Leonard et al.

APPLN. NUMBER : 10/031,152

FILED : January 14, 2002

FOR : Tissue Regenerating Agent

PRELIMINARY AMENDMENT

Hon. Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the application as follows:

IN THE SPECIFICATION

Page 1, after line 1, please insert --Background of the Invention--;

Page 2, after line 11, please insert --Summary of the Invention--;

Page 2, after line 15, please insert --Description of the

Preferred Embodiment--.

IN THE CLAIMS

Please delete claims 6-9. A marked-up copy of the amended claims is attached.

REMARKS

The above amendments were made to place the application into proper United States Patent Format.

Respectfully Submitted,

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Marked-up Amended Claims
10/031,152
March 20, 2002

1. Tissue regenerating agent comprising a fusion protein derived from a protein or a peptide sequence which effects uptake in cells and comprising a protein inducing the proliferation of cells.

2. Tissue regenerating agent comprising a fusion protein derived from the viral protein VP22 with a protein inducing the proliferation of cells.

3. Tissue regenerating agent comprising a fusion protein derived from a protein or a peptide sequence that effects uptake in cells, with the SV40 T-antigen.

4. Agent according to Claim 1, comprising a fusion protein derived from the viral protein VP22 with the SV40 T-antigen.

5. Agent according to Claim 1, comprising a fusion protein derived from the viral protein VP22 with a viral cyclin.

~~6. Use of the agent according to Claims 1-5 for regeneration of cardiac tissue damaged by an infarct.~~

~~7. Use of the agent according to Claims 1-5 for regeneration of nerve cells.~~

~~8. Use of the agent according to Claims 1-5 for cultivation of terminally differentiated cells.~~

~~9. Use of the agent according to Claim 8 for ex vivo cultivation, for example of cardiomyocytes with subsequent re-implantation.~~

Tissue regenerating agent

The invention relates to a tissue regenerating agent. The fields of application of this agent are medicine and the pharmaceutical industry.

A number of human tissues and organs essentially comprise terminally differentiated cells. These include, inter alia, the nerve cells of the brain and the cardiomyocytes of the heart. These cells are terminally differentiated, i.e. they no longer divide and cannot be induced to proliferate. This means that damaged or even dead cells cannot be replaced by proliferating, neighbouring cells, for example as in the healing of a wound. If, for example, in a blockage of the coronaries, cardiomyocytes are not sufficiently supplied with oxygen (heart infarct), the affected cells die off and are not replaced by new cardiomyocytes, but by fibrotic tissue, which can lead to drastic impairments of the function of the cardiac muscle. These coronary heart diseases are one of the most frequent diseases of the heart.

At present, there are no possibilities of therapy directly treating the causes, but merely attempts to limit the consequences of a heart attack. In severe cases, only a heart transplantation remains as the last resort. The objective of this invention is now to induce terminally differentiated cells to divide again, with the result that they can contribute to the regeneration of damaged, neighbouring tissue.

In principle, terminally differentiated cells can be induced to proliferate by tumour viruses. However, this unfortunately results in an irreversible transformation of the cells, i.e. a terminally differentiated and functioning cardiomyocyte is mutated into a cancer cell which grows uncontrolled, and in addition has lost the cardiac muscle

function. This approach is therefore not suited for therapeutic purposes.

Recently, a viral protein, VP22 from Herpes Simplex, has been described, which is exported from infected cells and taken up by neighbouring cells. The precise mechanism is not yet known. However, the transport process is independent of direct cell-to-cell contacts. Other proteins can also be transported when fused with the viral protein (Elliott G and O'Hare P (1997) Intracellular trafficking and protein delivery by a herpes virus structural protein. Cell 88: 223-233).

The objective of the invention is to provide a novel agent for the regeneration of tissue. It is based on the task of producing a fusion protein which induces the cells of damaged tissue to temporarily proliferate, thus effecting the regeneration of the tissue.

This task is solved in the measures portrayed in the claims.

The inventive tissue regenerating agent comprises an agent containing a fusion protein derived from a protein or a peptide sequence effecting the uptake in the cells, and a protein that induces the proliferation of cells. Preferably, a fusion protein derived from the viral VP22 protein with the SV40 T-antigen is used. On the basis of its varied functions, which cause cell proliferation and prevent apoptosis, the SV40 T-antigen is particularly well suited for this task. As an alternative, T-antigen related proteins or viral cyclins can be used. These cyclins include the K and V cyclins of the herpes virus. These cyclins are not inhibited by the cell cycle inhibitors of the cell and can thus induce proliferation without being impeded.

The second part of the task entails only temporarily inducing proliferation. After a few cell cycles, the cells are to return to the original terminally differentiated status and exercise their actual function. This task is solved with the inventive agent in that the agent is a protein which cannot replicate itself and is degraded by proteolytic enzymes. The stability of the fusion protein can be artificially amended as required by the inclusion of stabilising or destabilising peptides. This approach thus avoids irreversible genetic alterations and transformations of the cell, which would occur in DNA-based methods.

The use of this agent is done according to its purpose for regeneration of infarct-damaged cardiac tissue and for regeneration of nerve tissue damaged by injuries or disease. The agent is injected into the damaged areas and there taken up by the neighbouring cells. These cells are induced to proliferate, replace the dead cells and thus effect the tissue regeneration.

The agent is further also used according to its purpose for cultivation of terminally differentiated cells. Although terminally differentiated cells, e.g. nerve cells and cardiomyocytes, can be cultivated ex vivo, they do not proliferate and cannot be expanded for re-implantation or research purposes. The inventive agent is taken up by the cells following insertion into the culture medium and then effects the proliferation, i.e. multiplication of these cells. The dosage and duration of the treatment can be stipulated as required. After application of the agent has been stopped, the cells differentiate again and can either be re-implanted or used for research purposes.
It has been shown that the inventive agent can induce the S phase in terminally differentiated skeletal muscle cells (myotubes).

The invention is to be illustrated on the basis of a concrete example.

Example of implementation

The VP22 (UL49) gene is amplified with PCR from the Herpes Simplex Angelotti virus strain with primers which flank the open reading frame and remove the stop code. BamHI and XmaI restriction sites are added to the ends of these primers; with them, the PCR product can be cloned directly into an expression vector (pEVRF, Matthias et al., 1989). The T-antigen gene is amplified analogously from the SV40 DNA by means of PCR, and a XmaI and a XbaI restriction sites are added to the primers used. The PCR product is thus inserted into the expression vector at the C-terminal end of the VP22 gene. In the final cloning step, an oligonucleotide coding 6 histidine residues (His tag) and a stop codon is inserted at the C-terminal end of the T-antigen gene at the XbaI position. The final product is a fusion gene comprising the VP22 gene, the T-antigen and a His tag. The fusion gene is transcribed from the CMV promoter of the expression vector and translated from the translation signal of the Tk gene.

This expression vector is used to transfect COS-7 cells as described (Leonhardt et al., 1992). The fusion protein is exported from the producing cells into the medium due to the transport properties of VP22. The culture medium of the transfected COS cells conditioned in this way is continuously pumped via an affinity column (TALON, Clontech, Palo Alto, USA), which specifically binds the fusion proteins with a histidine tag. These affinity columns are used according to the instructions from the manufacturer. The fusion protein can then be eluted specifically with Imidazol and further purified by means of FPLC (ion exchange columns). The purified fusion protein is dialysed against normal saline solution and applied via catheters

directly into the cardiac muscle via the coronary arteries. As an alternative, the fusion protein can be injected directly and locally into the ischaemic cardiac muscle tissue.

Literature

Elliott G and O'Hare P (1997) Intercellular trafficking and protein delivery by a herpesvirus structural protein. Cell 88: 223-233

Leonhardt H, Page AW, Weier HU et al (1992) A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei. Cell 71: 865-873

Matthias, P, Müller M M, Schreiber E, Rusconi, S and Schaffner, W. (1989) Eukaryotic expression vectors for the analysis of mutant proteins. Nucl. Acids Res. 17, 6418

Patent Claims

1. Tissue regenerating agent comprising a fusion protein derived from a protein or a peptide sequence which effects uptake in cells and comprising a protein inducing the proliferation of cells.

2. Tissue regenerating agent comprising a fusion protein derived from the viral protein VP22 with a protein inducing the proliferation of cells.

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9. Use of the agent according to Claim 8 for ex vivo cultivation, for example of cardiomyocytes with subsequent re-implantation.

Norris, McLaughlin & Marcus, P.A.

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If each inventor understands English, the Declaration and Power of Attorney below is suitable for use when filing a regular patent application and also when entering the national stage, in the case of an International application designating the USA under the PCT.

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION	Attorney Docket No. 101195-70

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name,
I believe I am the original, first and sole inventor (if only one name is listed below at 201) or an original, first and joint inventor (if plural names are listed below at 201-205) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Tissue Regenerating Agent

the specification of which (check one)

____ is attached hereto

__✓__ was filed on ____12 July 2000_____

under Serial Number __PCT/DE00/02258__ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56.

I list below any prior foreign application(s) for patent or inventor's certificate in respect of which foreign priority benefits are claimed under 35 USC 119; and any prior foreign application(s) for patent or inventor's certificate in respect of which such foreign priority rights are not claimed and which has a filing date before that of any application in respect of which such foreign priority benefits are claimed:

Application Number	Country	Filing Date (day, month, year)	Priority Claimed under 35 USC 119
199 33 089.1	**Germany**	**15 July 1999**	YES: __✓__ NO: ____
			YES: ____ NO: ____
			YES: ____ NO: ____

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

Application No.	Filing Date

Combined Declaration and Power of Attorney
101195-70
Page 2

Customer No. 27387

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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	Post Office Address	City	State & ZIP/Country

205	Family Name	First Given Name	Second Given Name
	City of Residence	State or Foreign Country	Country of Citizenship
	Post Office Address	City	State & ZIP/Country

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 201	Date 15.01.02
Signature of Inventor 202	Date 15.01.02
Signature of Inventor 203	Date
Signature of Inventor 204	Date
Signature of Inventor 205	Date